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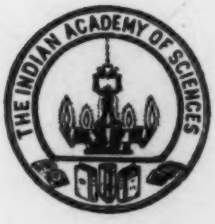

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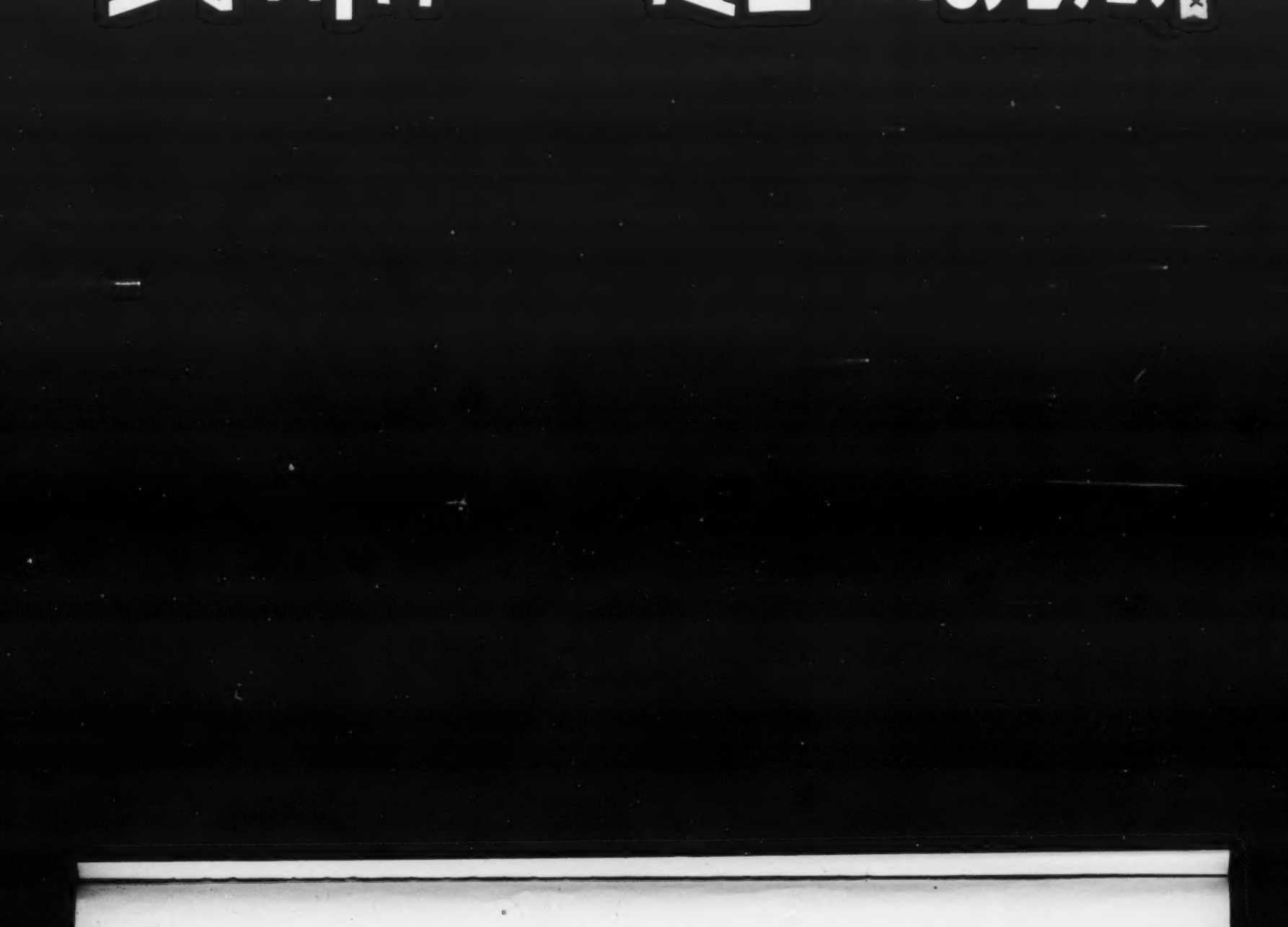

INDUCED RING-FASCIATION IN TOMATO PLANTS

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The phenomenon of Fasciation has been described under two main types: Band-fasciation and ring-fasciation. Band-fasciation is of wide-spread occurrence in the vascular plants (halophytes and hydrophytes are excepted), and it affects almost all their parts (Masters, 1869; Worsdell, 1915; White, 1948).

Ring fasciation is, on the other hand, rare in nature. Instead of becoming flattened as in band-fasciation the cylindrical main axis becomes enlarged and hollow " with the inner cavity somewhat freely exposed to the atmosphere " (White, pp. 326–27). White in his forty pages review of the phenomenon devoted only one small paragraph to ring-fasciation.*

Gorter (1951) and Wardlaw (1953) could induce ring-fasciation in tomato plants by the application of TIBA to their vegetative shoot apices. Their reports are illustrated with excellent sketches and photomicrographs. As this short paper has been prepared on the data provided by these illustrations I consider it necessary to give a short description of the latter.

Data

Gorter's Fig. 13 D (Pl. I) and photo. 4 A show the development of a ring-like out-growth (meristem) below the organic apex of the shoot after the application of TIBA. Figs. 5, 3 and 4 (Pl. II) show successive stages in the development of this outgrowth into a tubular ring-fasciated axis, and its resolution into a number of growth centres (when compared with the photo. 4 C they are flower primordia). Photos. 3 A and 3 B clearly show the changing over of the vegetative into flowering shoot apex on TIBA treatment. Photos. 4 B, 5, 6 and 7 show the internal structures of the treated axes in transverse (*t.s.*) and longitudinal (*l.s.*) sections. 4 B shows two treated seedlings in *l.s.*; 5, *t.s.* of ring-fasciated axis showing two rings of vascular tissues, the inner with inverse orientation with fairly advanced stage of tissue differentiation (*cp.* Worsdell, Fig. 26; and Wardlaw, Fig. 1). Photos. 6 is a *l.s.* of ring-fasciated axis with somewhat well-developed central bud,

* *Bot. Rev.*, **14**, 319–58.

and 7, a *l.s.* through young ring-fasciated axis resolved into separate growth centres (*cp.* Fig. 4, Pl. II).

Wardlaw's figures are all photomicrographs. Fig. 1 is a *t.s.* of the ring-fasciated axis at the level of the tip of the organic apex (central bud). It shows the hollow stem, two concentric rings of vascular tissues, the inner with inverse orientation, and the free tip of the apical dome; Fig. 2 is a *t.s.* of the same axis taken just below the organic apex; it shows three concentric rings of vascular tissues with normal orientation in the outer and inner rings, but inverted in the middle one. The inner ring is still in the provascular stage with xylem and phloem elements just differentiating. Wardlaw described the inner ring as belonging to the central bud. Fig. 3 is a *t.s.* of the same axis below the so-called ring-fasciated region showing the normal stem structure (herbaceous woody type).

A close examination of the figures and photographs described above bring out the following points: When TIBA is applied to the vegetative shoot apices of tomato plants (i) the growth and elongation of the apical dome is considerably slowed down, (ii) a ring-like lateral outgrowth takes place from the subapical region of the dome, (iii) the ring-like outgrowth then develops into a tubular axis enclosing the organic apex (Wardlaw), and resolved into a number of growth centres which ultimately develop into a ring-fasciated inflorescence (Gorter), (iv) the ring-fasciated axis shows two concentric rings of vascular tissues, the outer with normal and the inner with inverse orientation of xylem and phloem, (v) at the level of the origin of the tubular axis there occur three rings of vascular-tissues, the outer and inner with normal and the middle one with inverse orientation, and (iv) as a result of TIBA application the vegetative apex is changed into a flowering shoot apex (Gorter).

DISCUSSION

The whole problem may be discussed under the following two heads: (1) the method of origin of the ring-fasciated shoots in tomato plants under TIBA treatment, and (2) the origin and nature of the two and three concentric rings of vascular tissues in the ring-fasciated region and their absence lower down in the axis (Wardlaw).

1. *The method of origin.*—Since the days of Linnaeus those who made special studies of the phenomenon tried to explain the true morphology of the ring- and band-fasciated shoots. Linnaeus (1751) thought that fasciation of the axis was the result of an increase and fusion (congenital) of a large number of growth points or buds. Masters (1869) and Reed (1912) supported Linnaeus. Moquin-Tandon (1841) on the other hand considered

fasciation as the result of the flattening or enlargement of a single growing point. This was supported by Compton (1911) on anatomical grounds. Worsdell (1905) however, gave altogether a different explanation. According to him fasciation is the result of "a congenital fusion of an unusual number of branches fusing in one plane because of reversion to the ancestral form." With regard to ring-fasciation Worsdell thinks that it is an attempt on the part of the cylindrical organ to effect a multiple dichotomy (p. 90).

Nestler (1897) who made a special study of ring-fasciation suggested three possible ways of its formation, namely, (i) by the united growth of a large number of buds (growth points) as suggested by Linnaeus, (ii) by the formation of a ring of cells derived from the distal apical cell of the terminal bud, and (iii) as a result of the arrest of the growth of the distal region of the shoot apex followed by the continued enhanced growth of the embryonic tissue below (Wardlaw, p. 213). In the last two methods only a single growing point is involved (*cp.*, Moquin-Tandon).

Wardlaw supports Moquin-Tandon but figures and photographs of Gorter appear to support Linnaeus.

Schoute (1936) envisaged three superposed zones in the apical meristem each behaving biochemically differently but in correlation with one another. Wardlaw agrees with him and regards the formation of ring-fasciation in his material as due to the increased growth of the third zone and the arrested or very slow growth of the upper two zones of the shoot apex.

Cup-like limited outgrowth of the thalamus or torus is a common feature in the Disciflorеæ (Bentham and Hooker). One school of floral morphologists think that the carpel is formed by the outgrowth of the torus in the form of a rim-meristem, and particularly so in the case where the organic apex is converted into a basal placenta bearing the ovule (*cp.*, also the proliferation of the apex within the ovary). Wardlaw has cited a large number of workers who have reported increased growth of the subapical region of the apical dome under chemical treatment. Gorter's figures and photomicrographs are very convincing in this regard.

The development of a ring-like outgrowth (meristem) enclosing and outgrowing the organic apex under chemical treatment, and its final development into a tubular axis (Wardlaw) or into a ring-fasciated inflorescence (Gorter) may be regarded as quite an established fact [*see* Gorter's Figs. 5, 3, 4 (Pl. II) and photo. 4 C].

2. *Presence of two and three concentric vascular rings, one with inverse orientation of tissues* (Photos. Gorter's 5 and 6; Wardlaw's 1 and 2).—

Professor Wardlaw is a skilled Plant Surgeon, and whatever structural differentiation he notices in his surgical cases he regards them as prototypes of normal happenings in normally developing plants. He, therefore, believes only in the detached origin and basipetal differentiation of the provascular tissues in the shoot apices of vascular plants though there are many who have reported a continuous acropetal differentiation in their materials (Esau, 1954). The occurrence of double rings of vascular tissues, one with normal and the other with inverse orientation, he explains by saying that " pre-vascular tissue will be differentiated in relation to both the outer and inner flanks of the (same) ring meristem, and two vascular cylinders with their phloem oriented to the outer and inner surfaces respectively, will be formed " (p. 214). It is not supported by facts. In a recent review on the Primary Vascular Differentiation in Plants Esau (1954) has observed that " these deductions (drawn from surgical studies) are for the most part hypothetical because they are not based on critical histogenetic evidence " (p. 80).

The presence of the third vascular ring with normal orientation of tissues in Fig. 2 is explained by Wardlaw as originated from the apical meristem (*i.e.*, independent origin with basipetal differentiation). The absence of the inner and middle rings in Fig. 3 is explained by him (p. 212) as due to their fading out lower down in the axis without having made any connection with the stelar ring of the main axis, or in other words, their origin is detached and function perhaps undecided.

Gorter also holds similar views with Wardlaw on the origin of the outer and inner rings of vascular tissues in the ring-fasciated axis. She says that the prevascular tissue has its inception below the outer and inner flanks of the ring meristem, and collateral vascular tissue is differentiated in relation to both the outer and inner sides of the tube-like axis, *i.e.*, two separate vascular rings are seen in cross-section connected by only parenchymatous tissue (Photos. 5 and 7). Neither Gorter nor Wardlaw appears to have studied their ring-fasciated shoots in serial transverse and longitudinal sections and that alone would have shown the continuity or otherwise of the three concentric rings of vascular tissues.

Gorter and Wardlaw would not accept the presence of three concentric rings of vascular tissues as due to the invagination of the same stelar ring. Gorter's sketch 7 (Pl. II) and Photo. 4 B, as far as I can see, clearly show invagination of the axial stele. She writes to me (private communication) " I cannot see that in Photograph 7, which is a longitudinal section of the tubular axis, the inner and outer rings are continuous. These photographs have to give the final answer." That is very true. But when this photograph

is critically examined its very nature and structure show that it is an oblique section of the type of treated apex depicted in her sketches 3 and 4 (Pl. II). Moreover, a scrutiny of this figure under magnification indicates a continuity of the outer and inner vascular tissues through a provascular meristem over the central parenchyma in the right half of the photograph.

Neither Gorter nor Wardlaw referred to Worsdell (1915). Worsdell regarded ring-fasciation as " primarily induced by an apical invagination of the organ comparable to the turning inside out of a glove-finger " (pp. 90–91). He has illustrated his contention with two figures, one *t.s.* and the other *l.s.* (Fig. 26, p. 89). The *t.s.* shows two concentric rings of vascular tissues, the inner with inverse orientation, and the *l.s.* showing their origin by invagination of the original stelar tissues.

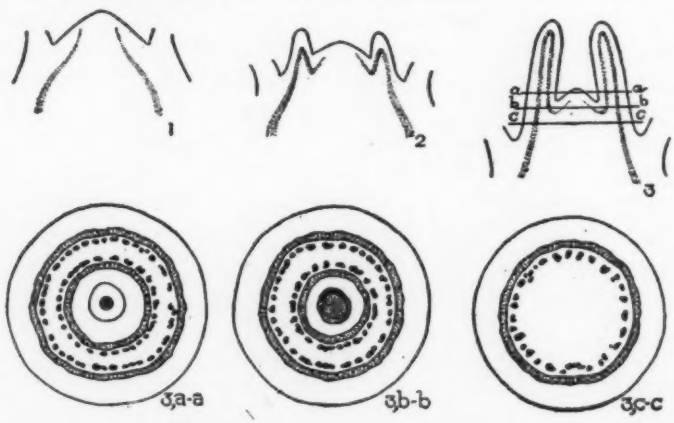
Both the authors also failed to take into their consideration the occurrence of similar structures showing double rings of vascular tissues with normal and inverse orientation of their elements in the receptacular ' floral cup ' of the inferior ovary reported in certain species of the Rosaceæ, Santalaceæ, Cactaceæ, and Calycanthaceæ (Puri, p. 512, 1951). These are cited as examples of the " Sinking of the gynoecium in the receptacle involving the invagination of the top of the stele " (Eames and MacDaniels, 1947, Figs. 167, K, L, pp. 352–53). Puri has given the following account of the manner in which the development of the cup and the invagination of the stele take place: The growing apex having become inactive growth is continued laterally in the region below the apex and vertically in the peripheral region round the apex. As a result the receptacle rises to a greater or less extent in the form of a hollow cup, carries the peripheral organs on its edge and leaves the carpels in the bottom. This intercalary growth also involves the procambial cylinder which as a result bulges out in the wall of the receptacular cup as a pouch. Obviously, therefore, the stelar bundles which have to supply carpellary traces appear to descend along the inner wall of the receptacular cup and have their xylem on the outside (p. 512). The receptacular cup with its double rings of vascular tissues, the inner with inverse orientation (*cp.*, Fig. 2 of Wardlaw and Photos. 5 and 6 of Gorter) is not considered as ring-fasciated axis.

The above comparison, however, does not account for the presence of a third ring of vascular tissue in the ring-fasciated axis of Gorter (Photo. 6) and of Wardlaw (Fig. 3). So far as the writer is aware only one case of ring-fasciation with three vascular cylinders was reported by Reichardt in a scape of the Dandellion (Worsdell, p. 90). Reichardt reported that the innermost ring ended blindly *passing from below upwards* in the solid core just below

the narrowed termination of the cavity, *i.e.*, in the organic apex (Gorter, Photo. 6)

On the evidence presented above the following explanation for the presence of all the three (never more than three in the reported cases) rings of vascular tissues in the ring-fasciated axes of Gorter and Wardlaw is suggested:

At the time when TIBA was applied to the shoot apices of tomato plants the provascular tissue had already reached the base of the apical meristem (continuous acropetal differentiation). It is on evidence that provascular differentiation extends to the flank meristem very close to, within 3 or 4 cell layers of, the extreme tip even before the visible emergence of a leaf primordium (Esau, 1951, 1954). The application of the chemical caused enhanced growth of the subapical region with the corresponding arrest of active growth or elongation of the apical dome. The growth of the subapical region initially took the form of a ring around the base of the free dome, which finally developed into a hollow axis with the slowly growing organic apex at its floor. This vertical growth also raised the provascular cylinder (main stele) in the form of a loop, its continuity with the apical meristem remaining unbroken (Figs. 1, 2 and 3). Gorter's Photos. 4B and 6 clearly show the continuity respectively of the outer and inner and the middle and the inner rings of the vascular tissues.



TEXT-FIGS. 1-3 are median longitudinal sections respectively of the shoot apices of tomato plants before and after the application of TIBA (all diagrammatic). Fig. 1. Shows the provascular tissues reaching the flank meristem before the application of TIBA. Fig. 2. Shows the sub-apical outgrowth and the raising of the provascular tissue along with it. Fig. 3. Shows the final position of the provascular meristem in the tubular axis. Figs. 3 *a-a*, *b-b* and *c-c* are *t.s.* through the last apex at different levels. These figures are the exact replica of Wardlaw's photomicrographs 1-3.

If the above interpretation is correct the nature and the fact of orientation of xylem and phloem in the three rings are easily explained, and the same prevascular tissue will appear in the three rings as reported by Gorter and Wardlaw in their materials (Figs. 3 *a–a*, 3 *b–b* and 3 *c–c*; *cp.*, also Eames and MacDaniels, Fig. 167, K, L). The growth of the apical dome is slowed down but never completely arrested (*see* Gorter, Photo. 6). The continuity of the provascular tissue is also never broken, but a portion of it may be left behind to mark its original position in the apical bud (Gorter, Photo. 6), and one is not to assume the origin of the three rings as independent of one another. This also explains the absence of the middle and inner rings in Wardlaw's Fig. 3.

Wardlaw concludes that " the vascular arrangements in these leafless ring-fasciation provide further evidence, of a rather novel kind, that the tissue pattern in the axis is determined by the shoot meristem " (p. 215). But the writer thinks on the other hand that it provides evidence for the continuous acropetal differentiation of the provascular tissue from the axial stele to the base of the apical meristem, as has been reported by Esau, Wetmore, Majumdar and others. Esau (1954) remarks that Wardlaw's conclusions are based on " weak foundation and the consequent hypothetical character of the guiding assumptions upon which the recent experimental work has been based " (p. 79).

The writer is of the opinion that Gorter's material is a real case of induced ring-fasciation of inflorescence axes (Photo. 4 C) like Reichardt's fasciated scape of the Dandellion. Gorter has further shown that application of TIBA causes a change of the vegetative shoot apex of tomato plants into flowering shoot apex (Photo. 3 A, B). The apical meristem of the flowering shoot resolves into a large number of flower primordia after the hollow tube has grown for some time [Figs. 5, 3, 4 (Pl. II) and Photo. 7]. Wardlaw's material on Gorter's more fuller evidence represents the lower portions of the ring-fasciated inflorescence axes before the flower primordia were formed. The anatomical evidence does not support any other conclusion.

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MARINE YEASTS OFF THE INDIAN COAST

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In contrast to the attention that has been lavished on terrestrial yeasts since early times, the yeasts associated with marine environments have not formed a popular subject for investigation. Even granting that marine microbiology is of recent development, it is difficult to understand why most investigators have persistently cold-shouldered this interesting group of organisms whilst the bacteria from similar environments have received perhaps more than their fair share of attention. The extent of our ignorance on marine yeasts is best summed up by quoting ZoBell (1946) who admits, " it is problematical whether certain of these organisms are indigenous to the sea because they occur so commonly in the air"! That a 'study of marine yeasts, using modern taxonomic methods, might yield interesting ecological data' has also been expressed by Mrak and Phaff in their recent review on yeasts (1948).

No doubt the occurrence of yeasts in the sea has often been reported, though usually as incidental to other micro-organisms. Fisher (1894 *a*), Fisher and Brebeck (1894), Issatchenko (1914), Nadson and Burgwitz (1931), and ZoBell and Feltham (1934) are some investigators who have reported the occurrence of yeasts along with moulds and bacteria in the sea. Our present knowledge on the role of yeasts in the sea will tend to be largely speculative, but they probably have an important part in modifying and preserving the marine environment.

This investigation, which to the best of our knowledge, represents one of the first attempts at understanding this neglected group of organisms, was undertaken to determine whether the sea has an autochthonous yeast flora, and if so, to characterise and establish the identity of the species usually encountered. The knowledge gained was also expected to have a utilitarian application in the control of marine foods vulnerable to yeast spoilage.

Methods and Media

It has been stated (Reuszer, 1933; Fred *et al.*, 1924) that the effect of the proximity of the land does not extend beyond about a mile from the

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open shore. Throughout these investigations therefore samples of sea water ensured to be free from terrigenous contamination were collected at a distance ranging from 2 to 6 miles off the coast. Sterile glass samplers were used for the purpose and when the samples could not be immediately worked upon they were stored at 0 to 5° C., but in no case was a sample worked upon which had been stored for more than 48 hours.

The isolation of yeasts from the sea water was best achieved by aerobic and anaerobic enrichment culture methodology. The following two media were routinely employed and were found to be very satisfactory: Both were made in a mineral base of pH 7 containing K_2HPO_4, 1 g.; NaCl, 20 g.; $MgSO_4 \cdot 7H_2O$, 0·1 g.; $FeSO_4 \cdot 7H_2O$, 0·02 g.; $CaSO_4 \cdot 2H_2O$ (saturated solution), 10 ml. and yeast extract (for vitamin requirements) 1 g. per 1000 ml. of distilled water. Whereas medium No. 1 was incorporated with 2% (w/v) glucose and $NaNO_3$, 0·05% respectively as the carbon and the nitrogen sources, the medium No. 2 contained 10% commericial sucrose and 0·05% $(NH_4)_2SO_4$ for the same purposes. The latter medium in conjunction with the use of anaerobic glass-stoppered bottles has been consistently observed to be well suited for the isolation of yeasts in general (Lobo *et al.*, 1953). The third serial enrichment when streaked over the same medium solidified with 3% agar yielded colonies of yeasts which were purified by restreaking several times or until colonies were as homogeneous in appearance as space relationship would permit. Lodder and Kreger-van Rij (1952) state that plating methods give practically the same guarantee of purity as making single cell or single spore cultures provided all colonies on the plate are uniform in type. Incubation in all cases was at room temperature (27 to 29° C.), this being compatible with the optimum for yeasts and the surface temperature of the ocean, which in tropical waters is usually reported to range from 28 to 30° C.

The media and methods employed for the characterisation of isolates were those currently favoured in the study of terrestrial yeasts, but the media were fortified with 2% NaCl to simulate the marine environment. The taxonomic procedures adopted for the identification was by resort to the recent system of classification suggested by Lodder and Kreger-van Rij (1952).

Morphological and growth characteristics.—The morphological features of yeasts have been used as important criteria in their classification and are undoubtedly of basic taxonomic importance. The general cell morphology and mode of reproduction were studied in purified and clarified malt extract (15 ° Balling) containing 2% NaCl. Observations on the nature of growth, pellicle and sediment formation, and the color, form, consistency and margin

of growth on malt extract agar were of differential value. The formation of arthrospores, pseudomycelia or true mycelia were studied on Rivalier and Seydel's medium (1932) which consists of peptone, 2 g.; glucose, 2 g.; NaCl, 2 g. and distilled water, 100 ml. This medium was found to be superior to the potato-glucose medium used for this purpose by some workers. Sporulation and the formation of ascospores have long been recognized to be of value in taxonomy and several media have been recommended for the purpose. The extensive work of Phaff and Mrak (1948, 1949) on sporulation has indicated carrot wedges to be well suited for this study and we found this medium to be satisfactory. Young, active, well-nourished cultures were inoculated on freshly autoclaved carrot wedges containing a small amount of NaCl. A vegetable juice agar (Wickerham, 1951) was also used with success for this purpose.

Physiological characteristics.—The morphological characteristics of yeasts in general are subject to the limitations of variation which are not infrequent in culture. Physiological characteristics as a rule are less variable and have been extensively used in yeast systematics. Fermentation characteristics, the assimilation of sugar, ethanol and nitrate, acid production and changes in litmus milk are some of the important physiological criteria exploited in species differentiation. The fermentation of glucose, maltose, sucrose, lactose, galactose, raffinose, melibiose and starch were studied in Durham tubes containing a basal inorganic solution containing also 0·1% yeast extract and 2% NaCl to which the separately sterilized carbohydrate solution was added to give a final 2% concentration. Results were recorded daily upto a period of 10 days. Assimilation tests were carried out in clean glass-ware and observing all precautions. The basal medium employed was the one suggested by Lodder and Kreger-van Rij (1952) and which has ammonium sulfate for the nitrogen source and a mixture of vitamins of the B complex family for the growth requirements. The various carbon sources were separately sterilized and added to this medium on the basis of 10 mg. of carbon per 10 ml. of the medium.

For assimilation of nitrate, 1% glucose was used as the carbon source and 0·078% of potassium nitrate as the nitrogen source. Results were recorded daily and after 1, 2, and 3 weeks for sugar assimilation, after 1 month for ethanol assimilation and after 1 week for nitrate assimilation. For the last mentioned test, a second set of tubes was also inoculated with growth from the first to minimize errors due to the presence of soluble nitrogen excreted by cells from the inoculum. This test was also supplemented by the routine biochemical tests for the reduction of nitrate recommended by the Society of American Bacteriologists (1944). The production of

starch was demonstrated by the formation of a blue color on the addition of a few drops of 0·02 N iodine to cultures grown in a medium recommended by Wickerham (1951). The browning of arbutin agar made in a nitrogen base synthetic medium containing 0·1% glucose denoted the splitting of this glucoside. Fat hydrolysis was studied by the appearance of clear zones around the growth after 10 days of incubation in Gorodkowa's tributyrin agar. The various changes brought about in litmus milk (modified by the addition of separately sterilized NaCl to give a final 2% w/v concentration) were also studied and found to be of some value. The inocula for all physiological tests were obtained from homogeneous sterile water suspensions of the organisms grown on yeast extract agar.

RESULTS

Altogether over 80 isolates of marine yeasts were obtained from 17 samples of sea water examined during the course of this investigation, of which 74 have been identified so far. Though none of these corresponded exactly to the species described in Lodder and Kreger-van Rij's (1952) treatise, the differences observed were only of a minor nature and related to the morphology and cultural characteristics which are known to be subject to variation.

Both the families *Endomycetaceæ* (ascosporogenous) and *Cryptococcaceæ* (non-ascosporogenous) were represented in our collection, but the majority of isolates were placed in the latter family. Thus only 18 isolates represented sporogenous yeasts, 10 being placed in the genus *Saccharomyces* and 8 in the genus *Debaryomyces* whilst the remaining 56 isolates belong to the genera *Candida* (30 isolates), *Torulopsis* (16 isolates), *Rhodotorula* (6 isolates), *Cryptococcus* (2 isolates) and *Trichosporon* (2 isolates), all representing the asporogenous yeasts. The general characteristics of these strains are outlined below.

Morphological and cultural characteristics.—The general morphological form consisted of round to oval cells occurring singly, in pairs, or frequently in clumps. The size varied from 1·4 to 8·4/9·8 microns. Vacuoles and refractile granules were invariably present. All isolates showed vegetative reproduction by budding. Most species grew moderately in solid and liquid malt extract media producing floccular or granular sediment in the latter medium. Uniform good turbidity with a pellicle typified growth in sucrose yeast extract broth and on the corresponding agar medium the colonies were usually round, smooth, convex, opaque, and moist or dry with an entire margin. Size of the colonies ranged from 0·5 to 3 mm. and colour from porcelain white to off-white except for the *Rhodotorula* which

produced pink, orange or red pigments. Colony characteristics in general cannot be said to be of much differential value.

Physiological Characteristics.—All the 74 identified isolates were non-acid producing and nonlipolytic, and were unable to utilise nitrate as the sole nitrogen source. Starch was synthesized by 1 isolate only and there was no marked reaction in litmus milk but for the production of alkalinity and reduction of litmus. The majority of the isolates gave luxuriant growth including a pellicle with ethanol as the sole source of carbon. The fermentation as well as assimilation of lactose and starch was in general poor. Glucose was assimilated by all isolates, the majority giving a luxuriant growth with a creeping pellicle.

The isolates made from the sea water and identified by the methods alluded to were the following species or near variants of them:

Saccharomyces :—S. steineri, S. fructuum, S. rosei.

Debaryomyces :—D. hansenii, D. nicotinæ, D. subglobosus, D. klæckeri.

Candida :—C. tropicalis (26 strains), *C. guilliermondi, C. melibiosi.*

Torulopsis :—T. glabrata, T. candida, T. famata.

Trichosporon :—unidentified strains.

In addition to these, a few unidentifiable strains representative of almost all the above genera have been isolated.

Discussion

The presence of several species of yeasts in association with a marine environment suggests that the sea has an autochthonous yeast flora. One of the most striking features of this marine flora is the relative predominance of asporogenous yeasts, an observation which is in agreement with that of the earlier workers (Fisher, 1894 *a*; Nadson and Burgwitz, 1931) inasmuch as those workers referred to them as Torula. If our collection may be regarded in any measure as representative of marine yeasts occurring off the Indian coast, the genus *Candida* may be singled out for special mention. Further, the regularity with which *Candida tropicalis* was encountered in the sea shows that it probably is one of the most common species of marine yeasts.

Yeasts have also been recovered from marine air (Fisher, 1894 *b*; McLean, 1918), fish nets (Freitas, 1953), and several varieties of marine foods (Hunter, 1920, 1922; Hanzawa and Takeda, 1931; MaCormack, 1950; Phaff *et al.*, 1952). The majority of informations incriminating yeasts as spoilage agents of marine foods point to the *Torula* which from all

available evidence appears to represent the most dominant of marine yeasts. Studies on other aspects of the physiology of marine yeasts with particular reference to their nutritional and salinity requirements (unpublished data) seem to suggest that the species described here have their origin in the sea. Furthermore, if one were to scrutinize the sources from which the species named above were originally isolated, one would be surprised to observe that most of them referred to have been derived from brined cucumber, salted beans, dates, grape must, fermented tobacco, palm wine, animal products, butter and human sources (Lodder and Kreger-van Rij, 1952) and this leads one to the speculation that in all probability the sea is the natural habitat of these yeasts.

ACKNOWLEDGEMENTS

The authors wish to thank the officials of the Taraporewala Aquarium, Bombay, for their assistance in securing the sea water samples and to Mr. V. Iyer of the Microbiology Department for his technical assistance.

SUMMARY

Microbiological analysis of samples of sea water collected off the coast of Bombay resulted in the isolation of over 80 yeasts by the enrichment culture methodology. A study of 74 strains indicated the preponderance of asporogenous over the sporogenous yeasts in the sea. The species *Candida tropicalis* probably occurs in the sea at all times and the other species belonging to the genera *Saccharomyces*, *Debaryomyces*, *Torulopsis*, *Cryptococcus*, *Rhodotorula* and *Trichosporon* have also been suggested as of marine origin.

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SPHACELOTHECA ON *ERIANTHUS MUNJA*

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Received August 7, 1954
(Communicated by Shree K. L. Khanna, F.A.Sc.)

Occurrence of smut caused by *Sphacelotheca* on *Saccharum* and allied genera namely *Erianthus*, *Narenga* and *Sclerostachya* is rather rare and the species reported so far was *S. schwienfurthiana* (Thum) Sacc., by Khanna and Ramanathan (1946) on *Erianthus munja*, by Chona (1951) on *Saccharum spontaneum* and by Panje (1952) on five forms namely SES 153, 185, 190, 195 and 240 of *Erianthus* out of 305 of wild *Saccharums* collected by him till 31-5-1952 from all over the country. The report of sugarcane smut caused by its another species [*S. cruenta* (Kuehn) Potter], by Chona and Munjal (1951) was not tenable because the host-varieties, *viz.*, Co. 560 and Co. 561, two out of a collection of nearly 600 varieties growing at Karnal, were back-crosses of *Sorghum halepense* on Vellai, a thick indigenous cane of Madras State (Sharma, 1953).

The present note deals with another kind of smut of *E. munja* (Form N/4 collected from Raini, District Muzaffarpur, in 1950) the causal agent of which, though belonging to this genus, was found, in several respects (Table I), to differ from *S. schwienfurthiana* occurring here on a form collected from Bhikna Thoree in submontane tract of district Champaran (*loc. cit.*, 1946).

While the Bhikna Thoree clumps throw out smutted arrows all the year round, the emergence of such arrows in N/4 is confined to the normal arrowing period of this species, *viz.*, November-December. As the clumps bearing these arrows in the latter form, get nearly two seasons to grow, they attain a height of 300–360 cm., which very nearly approaches that of normal stalks (Plate I, Fig. 1 *a*, *b*) whereas those of Bhikna Thoree form do not grow more than 90–120 cm. Apart from reduction in the height of the plant, the smutted arrows themselves were diminutive ($22 \cdot 5 \times 3 \cdot 7$ cm.) in Bhikna Thoree (Plate I, Fig. 2) those in N/4 being almost equal to healthy ones. The general appearance also of the smutted arrows in two forms under low magnification was so strikingly different as would appear from

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TABLE I

Sphacelotheca *on the two forms of* Erianthus munja

	Particulars	Form N/4	Bhikna Thoree
1.	Time of appearance of smut	Smutted arrows are formed only during the normal flowering season, *i.e.*, November-December	Smutted arrows emerge all the year round
2.	Height of culms with smutted arrows	300–360 cm.	90–120 cm.
3.	Average size of smutted arrows	48·8×11·3 cm. (more or less equal to the healthy ones)	22·5×3·7 cm.
4.	Average size*		
	(*a*) Sorus (i) Size	5·1×1·5 cm.	3·0×0·8 cm.
	(ii) Shape	Elliptical	Tubular; slightly curved and pointed
	(*b*) Columella	7·0 cm.	4·9 cm.
	(*c*) Anthers	Exserted	Held within the glumes
	(*d*) Spore:—		
	Minimum	6·0 μ	7·4 μ
	Maximum	10·6 μ	13·0 μ
	Greatest number	190 between 7·6 and 8·6 μ	153 between 10·0 and 11·0 μ
	Average	8·1 μ	10·4 μ
	Standard Error	$\pm$ 0·18 μ	$\pm$ 0·29 μ

* 4 (*a*) and (*b*) are average of 50 measurements while for 4 (*d*) 300 spores were measured with Ramsden Screw micrometer, one division on the drum being equal to 0·2μ.

Plate II, Fig. 3. The sori and columellæ in N/4 were similarly larger than those formed in Bhikna Thoree. But reverse was the case for the size of spores which was significantly different even at 1% level. As compared to the size of spores of the three species given by Chona and Munjal (1951) those formed on N/4 were almost equal to the spores of *S. schwienfurthiana* while the pathogen on the Bhikna Thoree form had the largest spores.

Though in both the cases, stamens were not involved in the formation of a sorus, their filaments in Bhikna Thoree form were short, stumpy and attached to sorus-wall at different levels, while in N/4 they were ribbon-shaped and fixed in their usual position, *i.e.*, below the ovary now forming a sorus. In the former, the anthers were held within the glumes whereas they were almost always exserted in the latter.

The spores of N/4-pathogen germinated readily in distilled water at room-temperature, *i.e.*, 70–80° F. and produced a four-celled promycelium on which sporidia were borne laterally on alternate cells (Plate II, Fig. 4). The sporidia were normal, *i.e.*, hyaline, single-celled and fusiform.

Specimens of these two pathogens were found to be identical by Dr. Lee Ling, Rome, and Systematists at the Royal Botanical Garden, Kew, and Indian Agricultural Research Institute, New Delhi. But they were not unanimous as to the species. Dr. Lee Ling preferred to call both *S. sacchari* and considered the variation in spore size a natural phenomenon in any group of living organisms. He also remarked that Indian workers called them by different names chiefly because of difference in spore size, *S. schwienfurthiana* for Bhikna Thoree collection and *S. sacchari* for the N/4 collection. At New Delhi they were identified as *S. schwienfurthiana.*

The modern trend in smut nomenclature against recognising species not clearly defined by morphological characters, naturally raises a question as to whether the two organisms can be so very different in their effects on the same host as these were found to be and still belong to the same species. In view of so very different host reaction, the possibility of these pathogens belonging to two different species cannot be easily brushed aside. This host-parasite relationship gets further complicated when one takes into consideration the presence of not only physiologic specialization in different species of *Sphacelotheca* noted by Tisdale *et al.* (1927) but also of clonal differences within the host itself, both morphological and physiological as recorded by Sharma (1952). It is somewhat easy to unravel the former by testing the two pathogens on a number of forms of the host. But for the latter there does not seem to be any way out because one clump is not likely to be similar to the other unless the two have a common origin. Because of the systemic infection of clumps by both the pathogens, the possibility by getting two clumps perfectly healthy for cross inoculations seems to be very very remote.

If the mode of infection in this case happens to be either at blossom (James and George, 1925) or at seedling stage (Kulkarni, 1922) as noted in *Sorghum*, the unstability of genetical make-up of *Erianthus* genus as a whole, due to the presence of high polyploidy, precludes all chances of disentangling the puzzle.

Further work is in progress.

The authors are indebted to Shree K. L. Khanna, Director, Sugarcane Research and Development, Bihar, for his keen interest in this work and

a *b*

FIG. 1. (*a*) Smutted arrows of N/4. (*b*) Healthy arrows of the same.

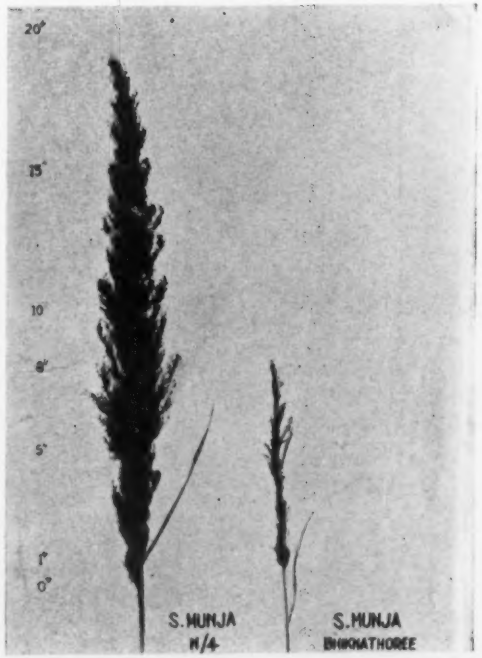

FIG. 2. Smutted arrows of two forms of *Erianthus munja*.

a *b*

FIG. 3. Parts of smutted arrows. (*a*) N/4. (*b*) Bhikna Thoree (×3)

FIG. 4. Germinating spore with sporidia formed alternately (×500).

Shree M. P. Jha, Statistician at this Station, for the statistical analysis of spore-measurements. The identification of the pathogens so kindly done by Dr. Lee Ling, International Agricultural Research Institute, Rome, and Systematists, Royal Botanical Garden, Kew, and Indian Agricultural Research Institute, New Delhi, is thankfully acknowledged.

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TWO NEW BOTHRIOCEPHALIDS FROM THE MARINE FISH *SAURIDA TUMBIL* (BLOCH.)*

By C. K. Subhapradha

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(Communicated by N. K. Panikkar, F.A.SC.)

1. Introduction

In the course of my studies on the cestode parasites of the fishes of Madras coast, it was found that the adult cestodes occur mainly among the elasmobranches while the larval cestodes occur mainly among the teleosteans. Only one teleostean fish, *viz.*, *Saurida tumbil* (Bloch.) was found to harbour two species of adult cestodes belonging to the family *Bothriocephalidæ* Blanchard. One of the species is of very common occurrence. It belonged to the genus *Bothriocephalus* Rudolphi. The other species was found only once. It belonged to the genus *Oncodiscus* Yamaguti, 1934. As both the species are new to science a detailed study of them has been made.

2. *Bothriocephalus penetratus* Spec. nov. †

(Figs. 1–5; Pl. III, Figs. 1–6)

Specific diagnosis

Head and part of the neck embedded in the liver of the host and enclosed in a thick sheath, in rare cases attached to the intestinal mesentery; strobila lying inside the intestine of the host; worms 48 to 108 mm. long, exclusive of the neck and head; over 300 distinct segments each giving rise to prominent wing-like expansions laterally; segmentation incomplete; testes 50 to 60 in number; cirrus pouch lying a little to the right or left of the segment; uterine pore oval or round, lying along the median longitudinal axis; vitellaria not extending to the median field; operculum not visible in the fresh eggs; parasitic in *Saurida tumbil*.

Description

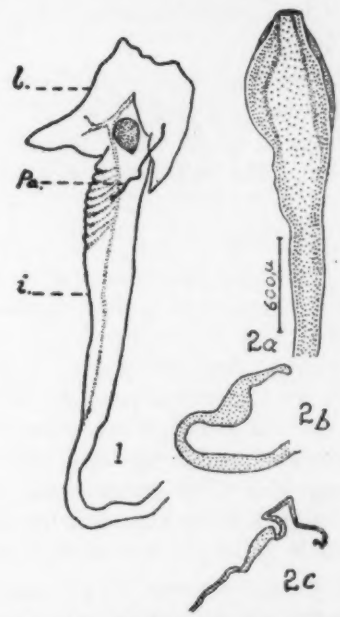
The most striking feature of this parasite is its mode of attachment to the host. The strobila of the worm lies inside the intestine of the host while the neck and head after boring through the wall of the intestinal cæcum

* Part of thesis approved for M.Sc. Degree of University of Madras in 1948.

† Was referred to as *B. pseudoscolex* in the thesis but a new name is here adopted.

(Fig. 1) pass along the mesentery to the liver and come to be lodged inside the latter (Pl. III, Fig. 2). The part that lies outside the intestine is enclosed in a thick tough sheath. In a transverse section this sheath is seen to be composed of two layers, an inner structureless layer which stains very deeply with Heidenhain's iron hæmotoxylin, and an outer layer consisting of irregularly scattered cells with granular contents (Pl. III, Figs. 3 and 4). It is quite probable that the inner layer is secreted by the parasite and the outer by the host.

In the adult worms the neck and the head have completely lost the power of motion, and due to the enveloping thick sheath, they are stiff and break easily. The head has undergone degeneration and is reduced to a



FIGS. 1 & 2. *Bothriocephalus penetratus*, sp.n.

Fig. 1. Intestine and liver of host showing the position of the parasite. Fig. 2 *a*. Head of a young worm, undegenerate. Fig. 2 *b*. Head, partly degenerate. Fig. 2 *c*. Head, completely degenerate.

thin long filament (Fig. 2 *c*), which is as much as 20 mm. in some specimens. It is of uneven thickness, but generally the basal portion is stouter than the apex which is usually pointed. The whole of the head region lies in close coils inside the sheath, which in this region is comparatively thin (Pl. III,

Fig. 3) and is enlarged into a bulb-like structure (Pl. III, Fig. 1). The neck has also undergone elongation. In some specimens it attains a length of 60 mm. Part of the neck region lies along the intestinal mesentery, while the rest is embedded in the liver, where it has a very tortuous course. Though the head and the neck have undergone degeneration in external structure, the tissues, however, do not show any degeneration. In a transverse section, the wall of the head and neck shows a structure similar to that of the body wall (Pl. III, Figs. 3 and 4).

In a few examples the head and the neck instead of being lodged in the liver remained attached to the mesentery round the intestine, but even here the degeneration is similar to that described above. In one instance two worms had penetrated the intestinal cæcum at the same point and the proximal parts of their necks were enclosed in a common sheath but their distal parts were separate.

The degeneration of the head seen in this species somewhat recalls the degeneration of the head seen in *Fistulicola plicatus* (Rudolphi), *Eubothrium rugosum* (Batsch) and some of the species of *Abothrium* Beneden. In these, however, the head is embedded only in the wall of the rectum or of the intestine.

The true nature of the scolex has been made out from three fairly young specimens. In one specimen the head had penetrated through the wall of the intestinal cæcum and was attached to the mesentery just above the cæcæ. The neck was short. The head was enclosed in a thin transparent sheath, within which it was expanding and contracting. It had not undergone any degeneration (Fig. 2 *a*). In the second case the head had penetrated the liver, but when teased out and placed in sea-water, it was moving actively. This also showed no degeneration. In the third, the head had just begun to degenerate. It had become slightly elongate and the anterior end had become pointed. The shallow grooves indicating the bothria had disappeared (Fig. 2 *b*). When placed in sea water it exhibited very little movement.

The neck probably elongates as the head moves forward. Inside the liver the head in some specimens appears to make a number of turns before it loses the power of motion and begins to degenerate. This would account for the tortuous course of the neck region inside the liver.

The true scolex consists of two shallow bothria (Fig. 2 *a*). The apex of the head is blunt. There is no apical disc. The scolex measures 1·4 mm. in length and 0·742 mm. in width at the middle, which is the widest region. Two larvæ of the same species were obtained from *Tichiurus haumela* (Forsk.). The larvæ also possessed no apical disc.

The mature worms measure from 48 to 108 mm. excluding the head and neck regions. The neck is distinct from the rest of the strobila. Segments are anapolytic. No eggs were obtained from the fæces of any of the fish.

In one instance the neck and head region of the parasite was obtained from the host attached to the liver, but there was no strobila in the intestine. Similarly on another occasion the neck and head region without the strobila was obtained along with two entire worms. This shows that the parasites when fully mature break away at the neck and pass outside the host entire, as with most other species of *Bothriocephalus*.

The posterior gravid segments extrude eggs when placed in sea-water. This added to the fact that no eggs are found in the fæces of the host probably indicates that the eggs are discharged after the parasite leaves the host.

The segmentation commences immediately behind the neck. The segments are at first indicated by transverse lines. The younger segments show a peculiarity in that the alternate ones are broader. This discrepancy disappears in the older segments. The segmentation does not extend to the centre, so that medially the segments are in direct continuation with each other. The sides of the segments give rise to prominent wing-like expansions which overlap the succeeding ones. There are over 300 segments. All are broader than long. The external divisions correspond with the internal segmentation. The width of the worm gradually increases till about the middle and then decreases again. Width of one of the worms at the middle was 1·43 mm. while at the posterior end it was 0·666 mm.

Body wall and musculature

The cuticle is very thin. Underlying that is a layer of sub-cuticular longitudinal muscle fibres. Below this is a layer consisting of scattered cells. The inner longitudinal muscles are well developed and form a thick layer. All the organs except vitellaria lie within the medullary parenchyma (Pl. III, Figs. 5 and 6).

Excretory system

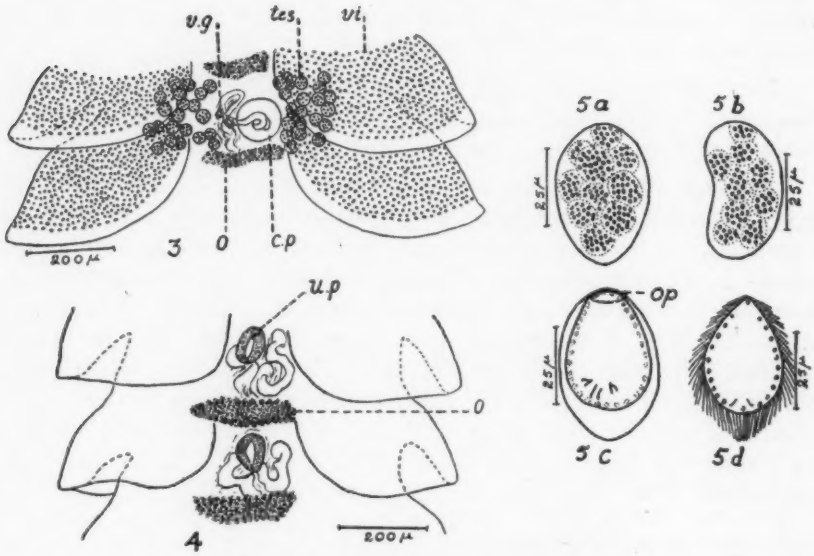
In sections of young segments 4 excretory vessels are seen on each side (Pl. III, Fig. 5) two of the vessels of each side being larger. In the older segments more than four vessels can be seen on each side.

Genital organs

There are 50 to 60 testes. They lie in two lateral fields in the medulla. The cirrus pouch lies a little to the right or left of the segment. The cirrus

lies coiled inside the pouch which appears round in whole mounts, and has a diameter of 0·13 mm. In transverse sections it is seen to be elongated in the dorso-ventral direction (Pl. III, Fig. 6). The cirrus pore is in the median line, lateral to the pouch. The vaginal opening lies by the side of and a little to the front of the cirrus opening (Fig. 3).

The ovary lies medially at the posterior end. It is at a level with the commencement of the wing-like expansion of the segment.



FIGS. 3–5. *Bothriocephalus penetratus*, sp.n.

Fig. 3. Mature segments showing the genital pores. Fig. 4. Segments showing the uterine pore. Fig. 5 *a*. Frontal view of the egg. Fig. 5 *b*. Lateral view of the egg. Fig. 5 *c*. Egg on the fourth day of development. Fig. 5 *d*. Newly escaped larva.

The uterine pore is oval in the immature segments (Fig. 4), while in the gravid segments it is round. The pore is situated in the middle of the segment a little above the level of the genital pores. The gravid uterus is oval encroaching upon the preceding segment. Its long axis coincides with the longitudinal axis of the body and occupies about $\frac{1}{3}$ the segment width.

The shell gland is seen in sections as a mass of cells round the oviduct.

Vitellaria consist of numerous small follicles lying in the cortex. They are confined to the lateral region and are most abundant in the wing-like expansions.

All other details regarding the genital organs are the same as for the genus.

The eggs are white at first but turn greyish black in colour within few hours. They are oval in one view and kidney-shaped in another view (Fig. 5, *a* and *b*). They measure 0·058 mm. by 0·33 mm.

It may be mentioned here that the eggs were left undisturbed in sea-water on two occasions to see if any development would take place. The embryos developed by the fourth day and the movements of the embryo within the egg could be seen. At this stage in some of the eggs the operculum was visible (Fig. 5 *c*). On the fourth night most of the eggs hatched out. A few hatched out on the 5th, 6th and 7th days. In all 95% of the eggs hatched out. The larva is of the typical hexacanth type (Fig. 5 *d*).

The structure of the scolax, the internal anatomy and the arrangement of the genital organs clearly show that these worms should be assigned to the genus *Bothriocephalus* Rudolphi. They however differ from most of the existing species in the absence of an apical disc, in the segmentation being incomplete, in the presence of a distinct neck and in the operculum not being visible in the fresh eggs. It may be mentioned here that Cooper (1918) in his monograph has stated that eggs of *Bothriocephalus* are non-operculate, but many authors have recorded the eggs as being operculate and in Wardle and McLeod's work (1952) the presence of an operculum is given as a generic character.

B. formosus Mueller and Van Cleave is also stated not to possess an apical disc. The present specimens differ from *B. formosus* in being larger in size and in the vitellaria being confined to the lateral regions. Testes in these specimens varies from 50 to 60, in *B. formosus* they are from 30 to 45. In *B. formosus* there is no degeneration of the scolex.

Bothriocephalus ganapatii Rao (1954) is stated to have a similar mode of attachment to that of the present species, but as the morphological and anatomical details have not been given a comparison between the two could not be made.

I have assigned the present forms to a new species *B. penetratus*. This species can easily be distinguished by its bell-shaped segments, with prominent lateral expansions, by the median uterine pores forming a straight line and its peculiar mode of attachment to the host,

Details of Collection

Date of Collection	No. of fish examined	No. of fish infected	No. of parasites obtained
3–10–45	1	1	2
29– 1–46	2	..	..
20– 9–46	4	1	3
4– 3–47	2	1	1
5– 3–47	1	1	3
10– 4–47	1	1	6
24– 7–47	2	..	..
30– 7–47	2	..	..
31– 7–47	2	1	4
6– 8–47	3	1	2
7– 8–47	3	1	2
27– 8–47	3	2	3
9– 8–47	2	2	4
11– 9–47	3	2	6
12– 9–47	2	1	2
11–10–47	2	1	1
1–11–47	1	1	1
3–11–47	2	2	3
7–11–47	2	1	2
8–11–47	2	2	9
10–11–47	2	2	4
18– 7–48	6	6	10
19– 7–48	3	2	4
25– 7–48	3	3	5

3. *Oncodiscus fimbriatus*, Spec. nov.

(Figs. 6–8)

Specific diagnosis

With the characters of the genus; worms measuring up to 68 mm. in length; bothria highly folded giving the head a globular appearance; terminal disc comparatively small measuring 0·5 mm. by 0·33 mm.; eggs oval, non-operculate.

Description

A single parasite was found lying loose in the intestine. Except for a slight contraction of the segments no other movements were noticed when the worm was placed in sea-water. This worm bears a striking resemblance to *O. sauridæ* Yamaguti (1934) in many of its external features and internal characters.

The head bears a superficial resemblance to *Anthobothrium variabile* (Linton, 1889) due to the margins of the bothria being highly folded. The terminal disc is small compared to the size of the head (Fig. 6 *a*). Each lobe of the terminal disc is 0·5 mm. wide and 0·33 mm. long. The hooks

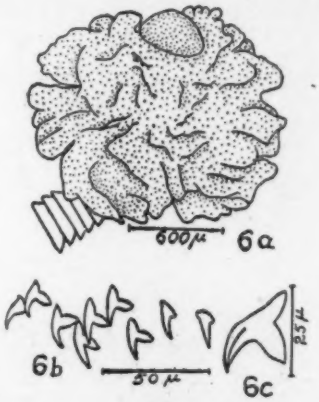


FIG. 6. *Oncodiscus fimbriatus*, sp.n.
Fig. 6 *a*. Scolex. Fig. 6 *b*. Hooks of the terminal disc. Fig. 6 *c*. Single hook of the same.

are 0·027 mm. long (Fig. 6, *b* and *c*). The head has a width of 2 mm. The median longitudinal furrow on the lateral surface described by Yamaguti in *O. sauridæ* could not be traced in the present specimen.

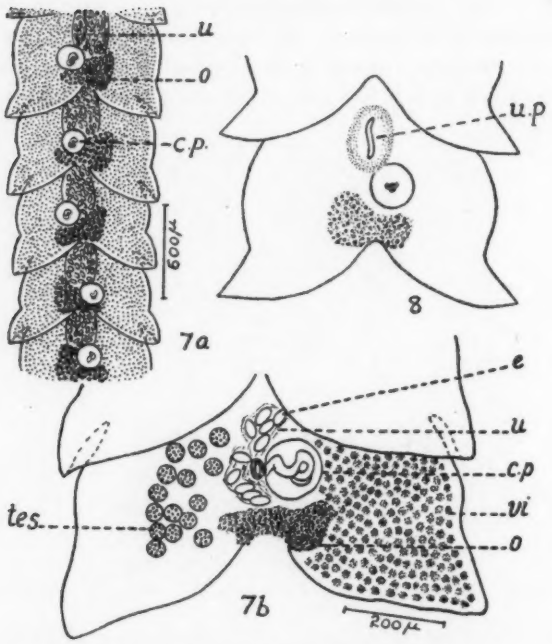
The entire length of the worm is 68 mm. The segmentation begins immediately behind the head. There are over 300 segments. They have an appearance similar to that of *O. sauridæ* (Fig. 7 *a*). They are all broader than long except the last few which are nearly as long as broad. The last segment measures 1·083 mm. in width and 0·83 mm. in length.

Excretory system

There are two excretory vessels on either side. At first these have a dorsal and ventral disposition. In the older segments they come to lie side by side. In the mature segments the excretory vessels are not clear.

Genital organs

There are up to 50 testes arranged in two lateral fields in the medulla (Fig. 7 *b*). The cirrus pouch lies a little to the right or left of the segment. It appears round in whole mounts. The course of the vas deferens outside the pouch could not be traced. The genital pore lies medially, lateral to the cirrus pouch. The vagina could not be traced.



FIGS. 7 & 8. *Oncodiscus fimbriatus*, sp.n.

Fig. 7 *a*. Part of the strobila showing the characteristic shape of the segments. Fig. 7 *b*. Mature segments. Fig. 8. Mature segment showing the uterine pore.

The ovary is transversely elongated. The two sides are not equal, the side opposite the cirrus pouch being more developed.

The uterine coils lie by the side of the cirrus pouch. In the gravid segments the uterine sac encroaches upon the preceding segment. It is oval and occupies about $\frac{1}{4}$ the segment width. The uterine pore is an elongated slit lying in the median line at the anterior end of the segment (Fig. 8).

The arrangement of the vitellaria is the same as in *O. sauridæ* Yamaguti.

As in *Bothriocephalus penetratus* sp.n., no eggs were obtained from the intestine of the host, even though most of the posterior segments were gravid. When the worm was placed in sea-water eggs were ejected. Eggs are oval, thin shelled and non-operculate. They measure 0·058 mm. by 0·033 mm.

This worm differs from *O. sauridæ* Yamaguti (1934) in the margins of the bothria being highly folded, in the absence of the median longitudinal furrow on the lateral surface of the bothria, in the fewer number of

testes, in the anterior position of the uterine pore and in the eggs being non-operculate. I have placed this parasite under a new species *O. fimbriatus*.

The generic characters of *Oncodiscus* should be emended to include forms without operculate eggs and with an anteriorly placed uterine pore.

A table of comparison between the two species of *Oncodiscus*:

	O. saurida	*O. fimbriatus*
1. Head	Margins slightly crenulate	Margins highly folded
2. Median longitudinal furrow on lateral face of bothria	Present	Absent
3. Testes	60–100	Up to 50
4. Uterine pore	About middle of proglottis	Anterior end of proglottis
5. Eggs	Operculate	Non-operculate

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EXPLANATION OF THE PLATE

Bothriocephalus penetratus, sp. n.

PLATE III

FIGS. 1–6. Fig. 1. Entire worm. Fig. 2. Section of liver of host with the parasite embedded in it. Fig. 3. T.S. of head region with the enveloping sheath. Fig. 4. T.S. of neck region with the enveloping sheath. Fig. 5. T.S. of anterior region of strobila. Fig. 6. T.S. of mature segment.

ABBREVIATIONS

c.l., cellular layer; *c.p.*, cirrus pouch; *e.*, egg; *e.v.*, excretory vessel; *h.t.*, host tissue; *i.*, intestine of host; *i.l.m.*, inner longitudinal muscles; *l.*, liver of host; *n.c.l.*, non-cellular layer; *o.*, ovary; *p.a.*, parasite; *sh.*, sheath; *tes.*, testes; *u.*, uterus; *u.p.*, uterine pore; *v.g.*, vaginal pore; *vi.*, vitellaria.

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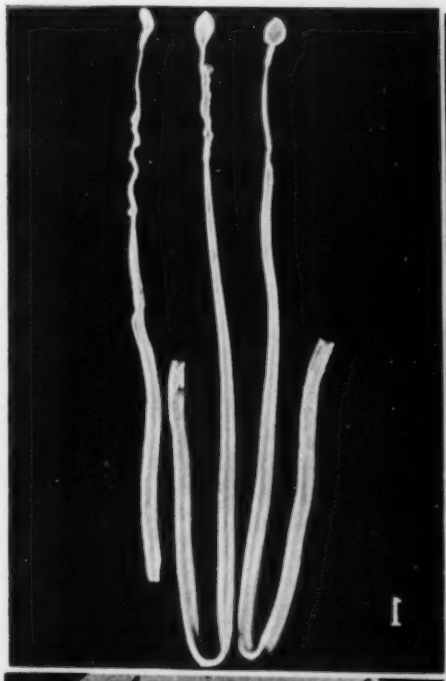

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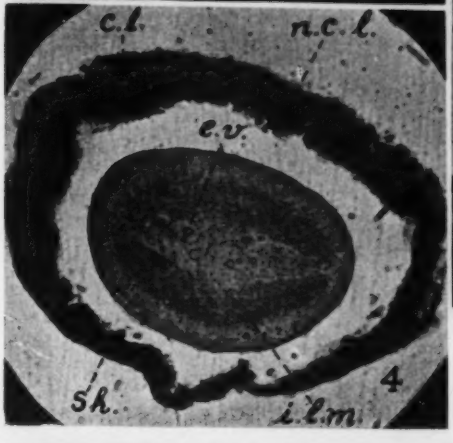
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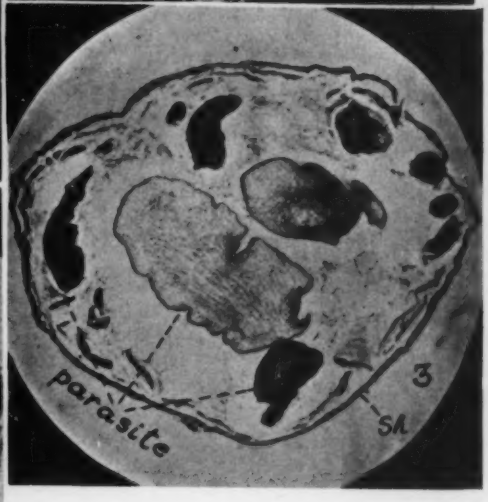
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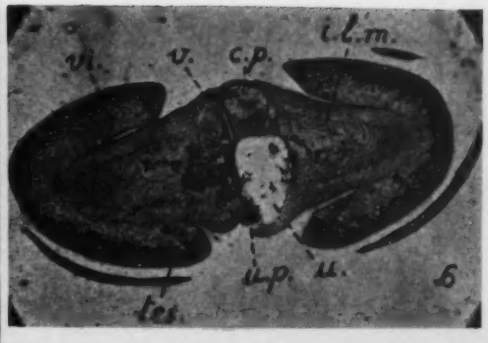
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MONOGENEA OF INDIAN FRESH-WATER FISHES

III. *Urocleidus notopterus* n.sp. (Subfamily Tetraonchinæ), from the Gills of *Notopterus notopterus* (Pallas), from Lucknow

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(*Research Scholar, Zoology Department, Lucknow University, Lucknow*)

Received August 25, 1954

(Communicated by Dr. M. B. Lal, F.A.SC.)

In earlier two communications (1952 *a*, 1952 *b*) I have described a new species and a new genus of the subfamily Tetraonchinæ, being the only representatives from the fresh-water fishes of India. The work was started in 1952 and the present communication is the third in the series dealing with a new species of the same subfamily.

Mizelle's (1936) refrigeration technique was followed. The host material was obtained from the fish market, Lucknow. All measurements except length and width of the body were made in glycerine mounts of fresh material. Curved surfaces were measured as straight lines extending between two most distant parts of the same.

Urocleidus notopterus N.SP.

A number of specimens were recovered from the gill filaments of a Feather Back, *Notopterus notopterus* (Pallas). Ten specimens of the host were examined and only seven were found infected, the infection was moderate and the number of ectoparasites recovered from one fish ranged from nineteen to thirty-six. The worms are easily recognised under a binocular microscope by the dense vitellaria spread over the body of the ectoparasite. The worms are soft, elongated and semi-transparent. The body consists of an elliptical anterior end, oblong body proper and a discoidal haptor.

An average sized worm (Fig. 1) is 0·38–0·42 mm. long, with width at the cephalic lobes 0·045 mm. and maximum body width being 0·07 mm. The cephalic lobes are not very well differentiated. There are four pairs of head organs and members of each side are fused together. The eye-spots are in two pairs, the anterior and the posterior pair, the latter with a larger number of melanistic granules. Pharynx is oval, 0·025×0·03 mm. Pharyngeal glands are situated on both sides of the pharynx and are made up of small globular glands. The outlines of the gut are obscured by the large amount of vitellaria spread in that region.

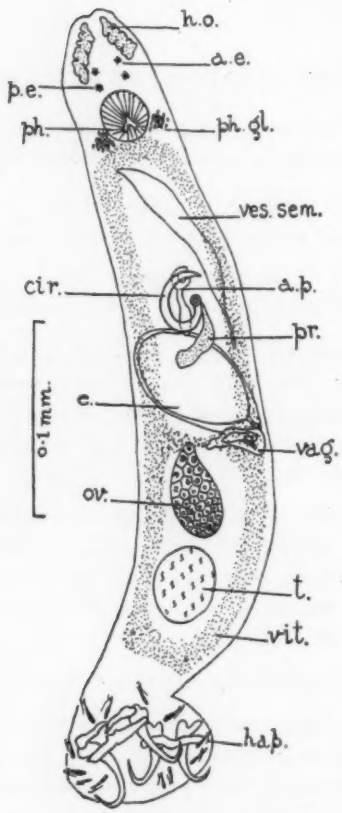

FIG. 1.

Haptor (Fig. 2) is discoidal, 0·085×0·065 mm. Peduncle is short and stout. The haptor is furnished with two pairs of anchors, a pair of bars, a pair of stout supporting bars and seven pairs of hooks. Anchors are almost similar in shape and size, differing only with respect to the bases. Each dorsal anchor consists of a stout rounded base with two or three indentations but there is no differentiation of a superficial and a deep root. Each anchor has stout shaft, fine recurved points and is winged. Each is 0·04–0·041 mm. in length (not following the curved surface) with width of the base 0·008–0·01 mm. The dorsal bar is a straight transverse shaft with dentitions in the middle anterior portion. Each is 0·04–0·041 mm. in length. Associated with the dorsal anchors are a pair of supporting bars. Each has a flattened proximal end and a conical distal end. The members

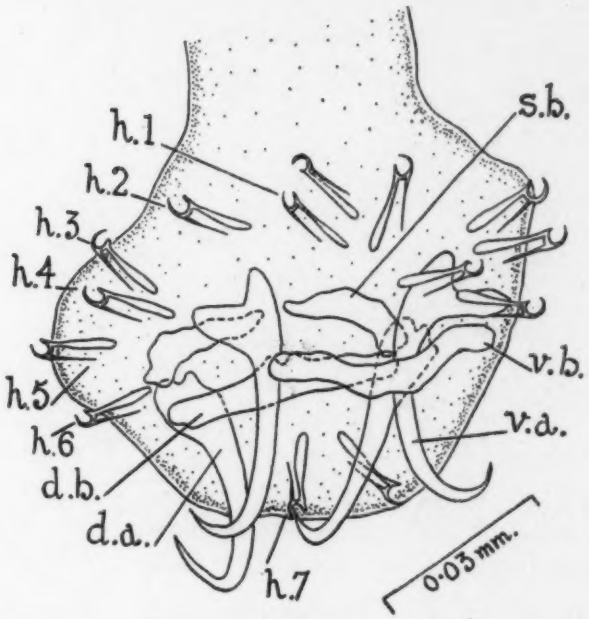

FIG. 2.

of the pair do not articulate with each other in the middle. Each is 0·022–0·024 mm. in length. Each ventral anchor consists of a deep bifurcate base, a stout shaft and fine recurved points. The base has a short blunt deep root and a similar superficial root. Each anchor is winged, 0·035–0·042 mm. in length (not following the curve) with width of the base 0·014–0·016 mm. The ventral bar is a transverse shaft with a depression in the middle and measures 0·035–0·037 mm. in length. The seven pairs of hooks are all similar in shape and size. The seventh pair lies at the base of ventral anchors. Each is sickle-shaped, consisting of a rounded oblong base, a short stout shaft, a sickle-shaped portion and a moderately long apposable piece. Each is 0·014–0·017 mm. in length.

Testis is median, oval. Vesicula seminalis is an elongated body terminating a short distance before ths pharynx. The copulatory complex (Fig. 4) consists of a stout tubular, coiled cirrus and a prong-like accessory piece, non-articulate at the base. The cirrus is thick-walled throughout its length and terminates in fine points. There is a large prostate gland terminating at the base of the cirrus. Cirrus is 0·055–0·06 mm. in length (following the curved surface) and accessory piece is 0·028–0·03 mm. in length. Ovary

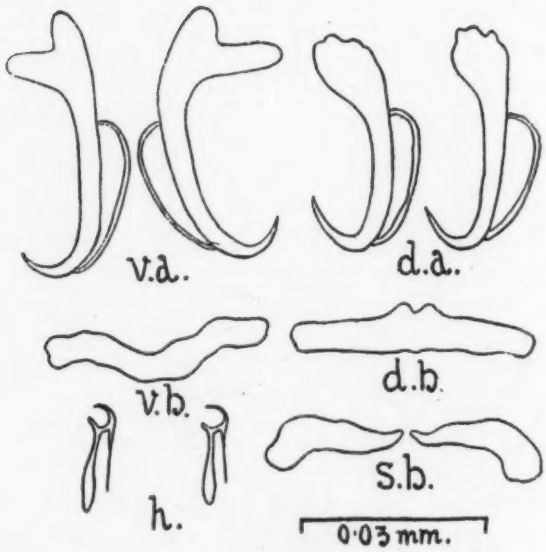

FIG. 3.

is median and pre-testicular. Receptaculum seminis not located. Vagina (Fig. 5) is dextral, highly chitinised with a large opening in it. Single egg (Fig. 5), oval with a short blunt projection, present near the vagina and enclosed in a semi-transparent uterus. It is 0·071×0·04 mm. exclusive of the length of the projection which is about 0·008 mm. in length.

Discussion.—The genus *Urocleidus* was created by Mueller in 1934. Mizelle (1938) redefined *Urocleidus sensu stricto*. Mizelle and Hughes (1938) redefined *Urocleidus sensu lato* with a key to the valid species. In the same paper they synonimised the Muellerian genera *Aristocleidus*, *Onchocleidus*, *Tetracleidus*, *Haplocleidus* and *Pterocleidus* with *Urocleidus*. Hargis (1952) revived *Haplocleidus* Mueller, 1937 by excluding *U. furcatus*, *U. parvicirrus*, *U. affinis* and *U. dispar* from the genus *Urocleidus* and assigning them to *Haplocleidus*. In the same communication Hargis also emended the characteristics of *Urocleidus* thus: "the dorsal and ventral anchors are equal or subequal in length and anchor bases are usually closely similar." The latter statement may not be strictly true.

There exist about 37 valid species of the genus *Urocleidus* to the present date, almost all of them being reported from North American fresh-water fishes. The present form while possessing the essential characteristics of *Urocleidus* Mueller, 1934, differs from all the known species in these charac-

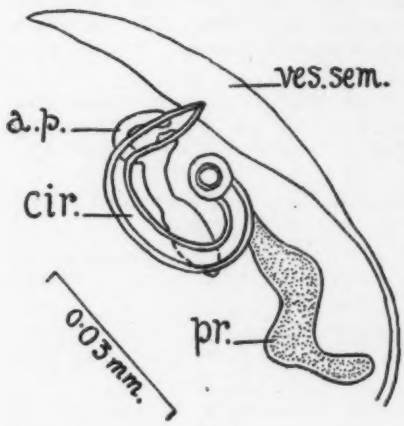

FIG. 4.

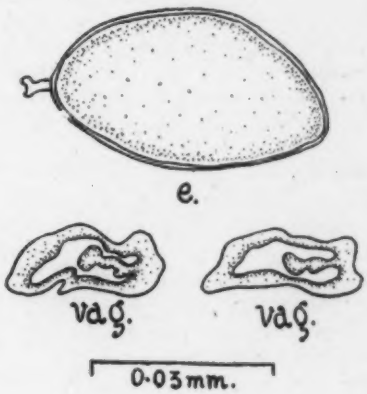

FIG. 5.

teristics: (1) Additional supporting bars in the haptor, (2) An enormous sized egg with a knob-like projection in the uterus, (3) Peculiar nature of the vagina, (4) Peculiar coiled, thick-walled cirrus and a prong-like accessory piece.

Taking into consideration the above important characteristics a new species of the genus *Urocleidus* Mueller, 1934 is created as *U. notopterus* after the name of the host. It is also interesting to note here that this is the first time when the genus *Urocleidus* is reported from India.

Host : *Notopterus notopterus* (Pallas).

Location : Gill filaments.

Locality : Lucknow, India.

ACKNOWLEDGEMENTS

The work was carried out under the guidance of Dr. M. B. Lal in the Department of Zoology, Lucknow University, Lucknow. I wish to express my sincere thanks to him for taking keen interest in the present work and also for allowing the use of his personal library. My thanks are also due to the Government of India for providing the scholarship to carry out the present studies.

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Explanation of Figures

Figs. 1–5. *Urocleidus notopterus* n.sp. Fig. 1. Entire worm (dorsal view). Fig. 2. Haptor. Fig. 3. Elements of the haptor. Fig. 4. Copulatory complex. Fig. 5. Egg and vagina.

a.e., anterior eye-spot; *a. p.*, accessory piece; *cir.*, cirrus; *d.a.*, dorsal anchor; *d.b.*, dorsal bar; *e.*, egg; *h.*, hook; *hap.*, haptor; *h.*1–*h.*7, hooks of left side; *ov.*, ovary, *p.e.*, posterior eye spot; *ph.*, pharynx; *ph. gl.*, pharyngeal glands; *pr.*, prostatic gland; *s.b.*, supporting bar; *t.*, testis; *v.a.*, ventral anchor; *vag.*, vagina; *v.b.*, ventral bar; *ves. sem.*, vesicula seminalis; *vit.*, vitellaria.

EMBRYOLOGICAL STUDIES IN THE COMPOSITÆ

I. *Launaea pinnatifida* Cass.

By J. Venkateswarlu, F.A.Sc. and (Srimathi) H. Maheswari Devi
(Department of Botany, Andhra University, Waltair)

Received September 6, 1954

Introduction

Compositae, the largest family of the flowering plants, comprising about 900 genera with over 1,300 species (Willis, 1948) enjoys a worldwide distribution. The majority are herbaceous and prefer open spaces while a smaller number are forest forms. A few are cultivated ornamental plants.

The family is of great interest to embryologists. As many as five different types of embryo-sac development, namely—Normal (Polygonum), Scilla (Allium), Peperomia, Drusa, and Fritillaria—occur within the family (Schnarf, 1931), besides some unclassified types in genera like *Balsamita vulgaris* (Fagerlind, 1939) and *Chrysanthemum cinerariæfolium* (Martinoli, 1939). The structure of the embryo-sac also presents some variations in the number of the antipodals, in the number of nuclei in them, and in the formation of antipodal and synergid haustoria in various members of the family. Polyembryony, apogamy and parthenocarpy are also known in some species. Apart from these interesting variations there is a great uniformity in the embryological features within the family.

In spite of extensive work in the family, some tribes have not received much attention and some aspects of embryology are rather poorly known. For instance, until recently a complete account of the detailed development of the embryo is only available in a few species like *Senecio vulgaris* (Souèges, 1920) and *Lactuca sativa* (Jones, 1927). Writing on this aspect Johansen (1950) says, "Despite a wealth of investigations on species belonging to the Asteraceae, the embryonomy of the family is in none too satisfactory a condition. The family, for one thing, is too large and unwieldy. Some taxonomists have segregated tribes within the family; others have split it into a number of separate families. If the latter procedure were accepted, it is believed that the embryology of the entire group could be established on a more satisfactory basis. There is only one completely detailed investigation (that on *Senecio vulgaris*)

based on modern embryological conceptions; certain of the older accounts, notably those of CARANO, indicate that there are significant minor departures which would warrant the segregation of several variations, from the accepted Senecio variation if the splitting into several families were followed. The whole problem requires more critical consideration than can be given to it in the present connection; moreover, certain of the groups (tribes or separate families) include no carefully investigated species". Since the publication of Johansen's book, an account of the embryo development in twenty-eight species distributed in twenty genera and representing seven tribes (Astereae, Heliantheae, Anthemideae, Senecioneae, Calenduleae, Cynarieae, Chorieae) is given by Vermin (1952).

Even so, the development of embryo in the tribes Helenieae and Heliantheae remains unknown. There remain several genera belonging to these and other tribes of Compositae growing in India in respect of which little or no embryological information is available. In the present studies it is hoped to work out the embryology of as many of them as possible.

The present paper deals with *Launæa pinnatifida* Cass., belonging to the tribe Cichorieae. Embryologically this tribe is better known than the rest in the family. Such interesting phenomena as apogamy (*Hieracium* and *Taraxacum*), polyspermy (*e.g.*, *Taraxacum* and *Crepis*) and polyembryony (*Crepis*) are known in this tribe (Maheshwari, 1950). *Launæa pinnatifida* grows wild on the sandy beach of Visakhapatnam and Waltair, and bears delightful yellow capitulae on which all flowers are ligulate.

Material and Methods

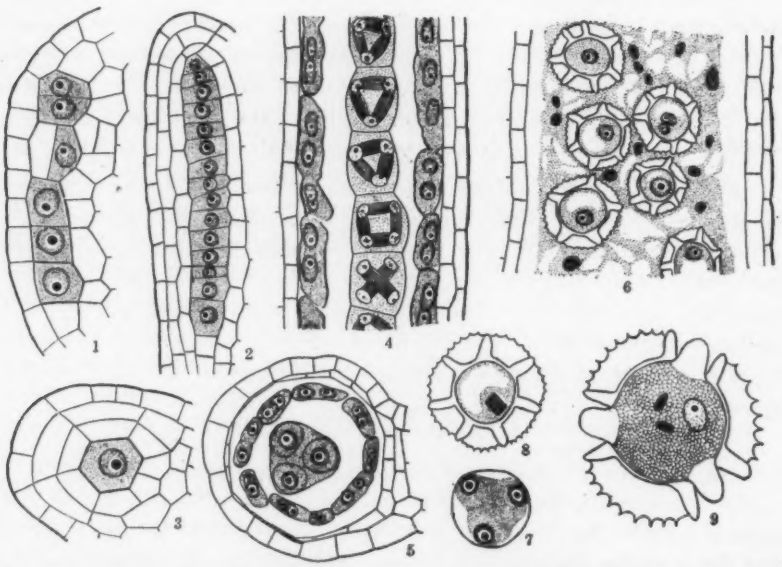
The material of *Launæa pinnatifida* was collected from plants growing at Visakhapatnam and fixed in Nawaschin's fluid and F.A.A. and sectioned following customary methods. Delafield's Hæmatoxylin was used for staining the sections. Some preparations on the basis of which the senior author (J. Venkateswarlu, 1939) published a note on the embryo-sac development in this plant have also been used.

Observations

Microsporogenesis and Male Gametophyte

In the very early stages of development each anther consists of a homogeneous mass of meristematic cells. The primary archesporium consists of a single hypodermal row of prominent cells which differentiates in each of the four lobes when the anther begins to show a four-lobed contour,

Soon the archesporial cells undergo a periclinal division (Fig. 1) giving rise to a primary parietal layer of cells to the outside and a layer of sporogenous cells to the inside. Once again the primary parietal layer divides periclinally to form two layers (Figs. 2 and 3). The inner of these layers directly becomes the anther tapetum while the outer divides periclinally and gives rise to two wall layers below the epidermis. Even in mature anthers no fibrous endothecium is differentiated in this plant. This has been verified by teasing the wall of the mature anther and examining the wall layers after clearing with chloral hydrate. Similar absence of endothecium was reported previously in *Oryza* (Juliano and Aldama, 1937), *Ditepalanthus* (Fagerlind, 1938) and *Balanophora* (Fagerlind, 1945).



FIGS. 1–9. Structure and development of the anther and pollen: Fig. 1. L.S. anther lobe showing a row of archesporial cells, × 707; Figs. 2 & 3. L.S. & t.S. anther lobes respectively showing single layer of P.M.C., and two wall layers beneath the epidermis, Fig. 2. × 527; Fig. 3. × 707; Fig. 4. L.S. anther lobe showing a single row of P.M.C., in II meiotic division. The radial walls of some tapetal cells have dissolved and there is only a single wall layer of cells under the epidermis, × 527; Fig. 5. T.S. of anther lobe showing a little later stage, × 527; Fig. 6. L.S. of anther lobe showing periplasmodium around the pollen grains. The nuclei of the periplasmodium are not in quite healthy condition, × 527. Fig. 7. Cytokinesis by furrowing in tetranucleale pollen mother cell, × 707; Fig. 8. Section of pollen grain showing first division of the nucleus, × 707; Fig. 9. Section of a three-nucleate pollen grain showing three germ pores in the exine, a large vegetative nucleus and two sperms, × 707.

The tapetum is a single layer of prominent cells which are binucleate (Figs. 4 and 5). The cells are at first uninucleate. Their nuclei undergo a mitotic division at about the time when pollen mother cell nuclei are in their prophase and the tapetal cells become binucleate. At about the time when uninucleate pollen grains are formed the inner and radial walls of the tapetal cells dissolve and the cytoplasm flows out into the anther lobe forming a periplasmodium (Fig. 6). The nuclei of the periplasmodium do not remain healthy for a long time but degenerate soon. Tapetal periplasmodium is found in *Blumea laciniata* (Banerji, 1942), *Eclipta erecta* (Bhargava, 1935) and many other Compositae (Schnarf, 1931). The periplasmodium diminishes as the microspores develop further and disappears finally in the mature anther.

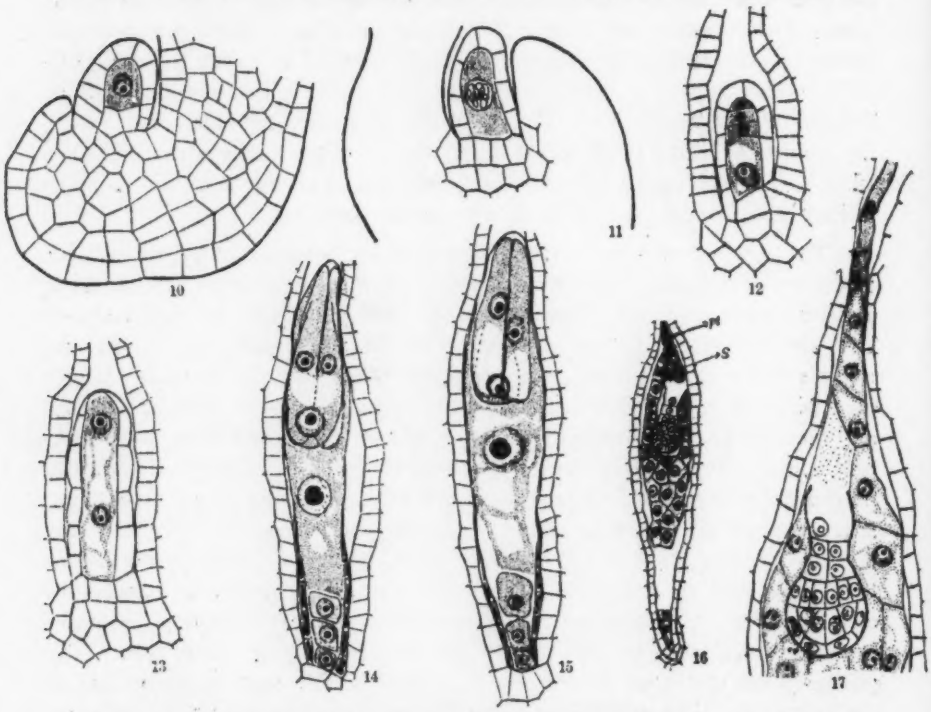
The cells of the inner layer arising from the first periclinal division of the archesporial cells divide transversely and only a single row of pollen mother cells is formed. The nuclei of these show one or two nucleoli. The division of the pollen mother cell is of the simultaneous type and meiosis does not show any unusual features. Cytokinesis is by furrowing (Fig. 7). Both bilateral and tetrahedral types of pollen tetrads are met with. The young 1-nucleate pollen grains are filled with cytoplasm, but soon a large central vacuole is developed and the centrally placed nucleus moves to a peripheral position. It divides there to produce a large vegetative nucleus and a small generative nucleus. The spindle has two similar poles (Fig. 8). This division is followed by the formation of a curved wall which demarcates a small lenticular generative cell, but the wall disappears soon and the generative nucleus migrates inwards and divides to give rise to two spindle-shaped male cells. The pollen grains are shed at this stage (Fig. 9). The mature pollen grains are spherical. The exine is echinate and has three germ pores (Fig. 9).

Megasporogenesis and Female Gametophyte

The ovule is anatropous, unitegmic and tenuinucellate. In young ovules with primary archesporial cell the integument is three to six celled thick in the basal region but in advanced stages of the ovule it is more massive. It does not contain any vascular trace such as known in some Compositae (Schnarf, 1931; J. Venkateswarlu, 1941).

The primary archesporium consists of a single conspicuous hypodermal cell (Fig. 10). This elongates and directly becomes the megaspore mother cell without cutting off a parietal cell (Fig. 11). A linear tetrad of megaspores is formed consequent upon the two meiotic divisions in it. The chalazal megaspore of the tetrad develops further and the three upper megaspores

of the tetrad degenerate (Fig. 12). The development of the embryo-sac is according to the Polygonum type. By the time the two-nucleate embryo-sac is formed the degenerating megaspores vanish completely (Fig. 13). The 8-nucleate embryo-sac grows rapidly and presses upon the nucellar epidermis



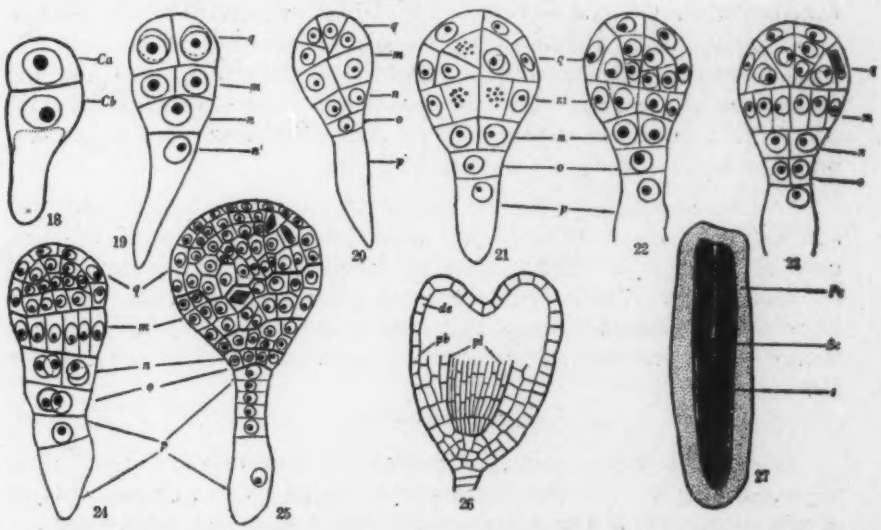
FIGS. 10–17. Structure and development of female gametophyte: Fig. 10. L.S. Young ovule showing a single hypodermal archesporial cell, × 527; Fig. 11. L.S. Ovule showing megaspore mother cell in its I meiotic prophase, × 527; Fig. 12. Part of ovule with linear tetrad of megaspores, chalazal one of which is functional, × 527; Figs. 14 & 15. L.S. of mature E-Sacs. Degenerating nucellar cells still visible, × 388; Fig. 16. L.S. E-sac with young embryo, cellular endosperm, persisting antipodals and persisting pollen tube. S= degenerating synergid mass, *pt*=pollen tube, × 129; Fig. 17. Upper part of the E-sac showing persistent pollen tube and an X-body, cellular endosperm and many celled embryo, × 340.

which is completely destroyed excepting at the basal region where the crushed nucellar cells can still be seen at the mature embryo-sac stage (Figs. 14 and 15). The cells of the innermost layer of the integument become differentiated as a glandular layer of cells with rich cytoplasm and large nuclei forming the integumentary tapetum such as found in other

investigated Compositae and Sympetalae in general. The cells of the integumentary tapetum are 1-nucleate. The embryo-sac is spindle-shaped and tapers towards either end. The cells of the egg apparatus are rather elongated. The egg cell shows the characteristic prominent micropylar vacuole and basally placed nucleus. The synergids are without hooks and show nuclei above the basal vacuoles. The antipodals are three in number and are uninucleate (Figs. 14 and 15). They persist for a long time (Fig. 16).

Fertilisation, Endosperm and Embryo

Fertilisation is porogamous. Only one pollen tube enters the ovule and it persists for a long time. Fig. 15 shows a case of an ovule with a persistent pollen tube and in this ovule a many-celled embryo is already developed. The pollen tube shows a small apical pore which is formed to facilitate the discharge of its contents into the embryo-sac. Endosperm is of the cellular type (Figs. 16 and 17). The primary endosperm nucleus divides earlier than the fertilised egg. In very old ovules the endosperm is completely consumed by the growing embryo.



Figs. 18–27. Various stages in the development of the embryo: *ca* & *cb*=apical and basal cells of the two-celled proembryo; *q*=Quadrants derived from *ca*; *m*=Upper daughter cell arising out of the first division of *cb*; *n*=Upper daughter cell derived from *ca*; *n'*=Lower daughter cell derived from *ca*; *o* & *p*=Upper and lower daughter cells derived from *n'* of which *p* forms a row of suspensor cells; Fig. 18, 20–25, × 527; Fig. 19. × 291; Fig. 26. × 172; Fig. 27. L.S. of cypsela, × 24; *pc*=pericarp; *sc*=Crushed remains of the seedcoat, *e*=embryo.

The first division in the fertilised egg is transverse (Fig. 18). Consequently a proembryo of two superposed cells, the terminal *ca* and the basal *cb*, is formed. The former divides by a vertical wall and the latter by a transverse wall to form a T-shaped tetrad. The derivatives of *cb* may be designated as *m* and *ci*. The former divides by a vertical wall and the latter by a transverse wall (Fig. 19). Thus *ci* later gives rise to a superior daughter cell, *n*, and an inferior one, *n'* (Fig. 19). The latter further divides transversely forming two superposed cells *o* and *p* (Figs. 20 and 21).

The terminal cell *ca* first undergoes two vertical divisions in which the walls are formed at right angles to each other producing quadrants in the tier *q* (Fig. 20). The four cells of the tier *q* divide by obliquely oriented walls (Figs. 20 and 21) and form octants. The cell *m* undergoes two vertical divisions at right angles to each other (Fig. 21). The cell *n* also divides similarly. In the tiers *q* and *m* periclinal divisions take place demarcating the dermatogen (Fig. 22). Later on further divisions take place in these tiers and periblem and plerome become differentiated (Figs. 24 to 26). The derivatives of *q* give rise to the cotyledons and stem tip and those of *m* to hypocotyledonary region and plerome initials of the root. The cells *n* and *o* undergo two vertical divisions at right angles to each other (Figs. 23 to 25). Their derivatives contribute to the formation of the root tip, root cap and dermatogen of the root. The cell *p* undergoes a few transverse divisions and forms a uniseriate suspensor of six cells. The lowest cell of the suspensor is larger and slightly vesicular (Fig. 26).

In *Launæa pinnatifida* the first division of the fertilised egg is transverse and both the terminal cell *ca* and the basal cell *cb* contribute to the development of the embryo proper. Thus the embryo development conforms to the Asterad type. The cell *m* (superior daughter cell of *cb*) gives rise to the entire hypocotyledonary region and plerome initials of the root and this feature is characteristic of the Senecio variation of the Asterad type (Johansen, 1950).

FRUIT AND SEED

In a fully developed seed the endosperm is completely absorbed. Even the seed-coat is almost completely destroyed except for the outermost layer of cells or two which remain in a crushed state between the embryo and the pericarp (Fig. 27).

SUMMARY

The development of the anther and pollen, megasporogenesis, female gametophyte, fertilisation, endosperm and development of the embryo of *Launæa pinnatifida* have been studied.

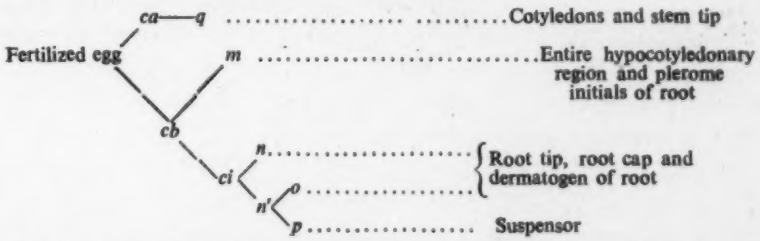
The primary archesporium in the anther consists of a single row of hypodermal cells. The structure of the anther shows epidermis, two wall layers, amœboid tapetum and a single row of spore mother cells. No fibrous endothecium is differentiated. The division of the pollen mother cell is simultaneous. Cytokinesis is by furrowing. The mature pollen grains are three-nucleate. The exine is echinate and has three germ pores.

The ovule is unitegmic, tenuinucellate and anatropous. An integumentary tapetum is differentiated. The primary archesporium is one celled. It directly becomes the megaspore mother cell without cutting off a parietal cell. A linear tetrad of megaspores is formed. The chalazal megaspore of the tetrad is functional. Embryo-sac development is according to the Polygonum type. Three persistent one-nucleate antipodals are formed.

Fertilisation is porogamous. The pollen tube is persistent.

Endosperm is cellular.

Embryo development takes place according to the Asterad type and keys out to the Senecio variation. Details of development are shown in the following schematic representation:



The seed is exalbuminous. Even the seed-coat is completely crushed by the embryo except one or two outer layers.

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